PCT/GB 2003 / 0 0 4 6 7 2

19 DECEMBER 2003

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 5 December 2003

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Patents Act 1977
(Rule 16)

The **Patent Office**

THE PATENT OFFICE
A
29 OCT 2002
RECEIVED BY FAX

290CT02 E591429-1 D02006
P01/7700 0.00-0225060.3

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1.	Your reference	JED1043
2.	Patent application number *(The Patent Office will fill in this part)*	0225060.3
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Astron Clinica Limited The Mount Toft Cambridge CB3 7RL
	Patents ADP number *(if you know it)*	7891427002
	If the applicant is a corporate body, give the country/state of its incorporation	England
4.	Title of the invention	Methods and Apparatus for Measuring Tissue Histology
5.	Name of your agent *(if you have one)*	Barker Brettell
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	St John's Innovation Centre Cowley Road Cambridge CB4 0WS
	Patents ADP number *(if you know it)*	07442494004

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of Filing *(day/month/year)*

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day/month/year)*

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	Yes

Patents Form 1/77

Patents Form 1/77

Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	7
Claim(s)	
Abstract	
Drawing(s)	7 only first

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: Barker Brettell
Barker Brettell

Date: 29.10.2002

12. Name and daytime telephone number of person to contact in the United Kingdom: Julie Dunnett — Tel: 01223 411355

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 01645 500505
b) Write your answers in capital letters using black ink or you may type them.
c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
e) Once you have filled in the form you must remember to sign and date it.
f) For details of the fee and ways to pay please contact the Patent Office.

Methods and apparatus for measuring tissue histology

This invention relates to methods and devices for the non invasive measurement of epithelial tissue histology and is particularly, but not exclusively, concerned with measuring skin histology. These epithelial tissues include the respiratory tract, the genital tract, the gastrointestinal tract and the retina of the eye.

The distribution of chromophores within these tissues and their lesions is considered to be potentially useful information for the screening of patients to identify those who should be referred to an appropriate clinician for further diagnosis through tissue biopsy, or other conventional tests. Devices to detect these distributions with non invasive techniques are therefore beneficial. Further, the simplification of such devices and the automation of the diagnostic process will make such devices available to wider populations.

The present invention improves the measurement technique described in GB 9624003.1 and related patents, and PCT/GB00/02124. The devices described in this application are improvements of those described in PCT/GB01/01986, PCT/GB01/03011 and GB0112501.2.

Figure 12 shows a cross section of skin. The major structural layers are well known, cornified layer 45, epidermis 46, dermo-epidermal junction 47, papillary dermis 48, reticular dermis 49. In normal skin melanocytes 50, the cells responsible for the production of the pigment melanin, lie in the epidermis. In some benign skin lesions, such as the compound nevus melanocytes can exist in a characteristic pattern in both the epidermis and the papillary dermis. In melanoma the melanocytes can be asymmetrically distributed in both the epidermis and papillary dermis.

The present invention provides additional information on the distribution of the pigment melanin in the papillary dermis, and includes a method for analysing this data automatically. This method is also applicable to other epithelial tissues with similar layered structures with differing optical properties.

Method to analyse presence and depth of chromophores in tissue

The equation described in GB application 9624003.1 page 10 lines 9 & 10 defines the expected colouration of human skin exhibiting the dermal descent of melanin as:

$$P_n(\rho_{mf}, \rho_{bl}, d_{mel}, d_{bl}, d_{l1}, d_{l2}, \Phi, d_m) = \int_0^\infty R_{2skin}(\lambda, \rho_{mf}, \rho_{bl}, d_{mel}, d_{bl}, d_{l1}, d_{l2}, \Phi)\theta(\lambda, d_m)^2 S(\lambda) S_{r_n}(\lambda) d\lambda$$

(Equation 1)

The terms of this equation are defined in GB application 9624003.1 pages 6 to 10. The equation may be modified to suit other tissues and chromophores and in the following

description the terms 'skin' and 'melanin' represent a particular example application of this method. The technique is particularly applicable to the chromophore haemoglobin in the skin where this method can be readily applied.

In the example of measurement of the location of melanin in the skin this equation is plotted in figure 1 with two primaries P1 & P2 chosen such that blood concentration is constant, and melanin can vary both in concentration and location in the skin.

Point 1 is the position of infinite melanin concentration in any position in the skin, point 2 is the point of no melanin in the skin. The line 5 connects points of increasing melanin concentration in the epidermis 45.

Figure 12 shows that that there are three variables of interest that define the distribution of melanin in the skin. The concentration of melanin in the epidermis, the concentration of melanin in the papillary dermis and the location of melanin in the papillary dermis. Two simplified cases are considered, the first where the concentration of melanin in the papillary dermis is assumed to be uniform, and secondly that the depth of dermal melanin is either zero or the depth of the papillary dermis itself.

Standard dermal melanin concentration case

In figure 1 point 3 is a point of chosen melanin concentration in the epidermis. As dermal melanin depth is increased (at standard concentration) the line 6 is generated. Point 4 represents the maximum possible depth that is the depth of the papillary dermis itself. This depth may have been standardised by the data calibration steps described in GB 9624003.1. Figure 3 shows the effect of repeating line 6 with different amounts of dermal melanin. Lines 5,10,11,12 are then plotted joining points on these lines where the same dermal melanin depth is used. Lines 5,10,11,12 define areas 7,8,9 that represent areas where colours of shallow, medium and deep dermal melanin depth will lie.

Standard dermal melanin depth case

Figure 4 shows the same axes, curve 5 with end points 1 & 2 as described above. In this case a point of epidermal melanin concentration is chosen 13 and two lines of low and high dermal melanin concentration are plotted with increasing depth 17, 16. The points at which the maximum dermal melanin depth is reached (papillary dermis depth) on these lines are 14 and 15.

Figure 5 shows these points 14, 15 joined to form lines of constant dermal melanin concentration at maximum depth 18,19,20. These lines bound areas 21,22,23 that represent areas where colours of low, medium and high dermal melanin concentration will lie, assuming dermal melanin exists and it's maximum possible depth.

It can be seen from this analysis that a single point on these two axes is insufficient to fully define the dermal melanin depth, dermal melanin concentration and epidermal

melanin concentration. The following invention overcomes this problem and allows a clinically useful measurement to be made.

Using actual values of melanin concentration in the epidermis, melanin concentration in the papillary dermis and dermal melanin depth that typically occur in human skin and in skin lesions, it was observed that areas 21,22,23 in figure 5 and the areas 7,8,9 in figure 3 were similar even though they were plotted using different simplification assumptions. These areas represent similar values of a fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). This function can also be interpreted as an approximation to a measure of 'dermal melanin volume'. This approximation can be intuativly understood by considering figure 5 and figure 3. Line 5 represents tissue with no dermal melanin. Areas 21 and 9 represent tissue with either deep, low concentration melanin, or shallow high concentration melanin (approximating to tissue with a low 'dermal melanin volume'). Whereas areas 23 and 7 represent tissue with deep, high concentrations of dermal melanin (approximating to tissue with high 'dermal melanin volume'). Line 20 represents the spectral response of tissue where significant melanin has penetrated the papillary dermis. 'Dermal melanin volume' is clinically significant because in a typical melanoma lesion the melanin producing cells, melanocytes, are similar because they are all produced by reproduction from a single mutated cell, and therefore changes in melanin concatenation are likely to be small compared to changes in the location of these cells. Therefore in such lesions areas with increased 'dermal melanin volume' area areas with increased dermal melanin depth. Dermal melanin depth is a well-known diagnostic and prognostic indicator in melanoma. If 'dermal melanin volume' is measured at an array of points across an area of skin, the measurements can be mapped to colours and displayed as a visualisation approximating to a map of dermal melanin depth across the lesion. This can assists the clinician is determining the degree of chaos in the distribution of melanocytes within the lesion.

A particular technique to make measurements of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) or 'dermal melanin volume' is as follows. A spectral measurement is made using a device as described in figures 8,9 & 10 or devices described in PCT/GB01/01986 or PCT/GB01/03011, and the measurements plotted on a graph indicating the areas of similar values of fn(Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration) (figure 6). This measurement can then be categorised by fn(Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration).

A second particular technique to make measurements of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) or 'dermal melanin volume' is as follows. A spectral measurement is made using a device as described in figures 8,9 & 10 or devices described in PCT/GB01/01986 or PCT/GB01/03011 and plotted on the graph shown in figure 5. This measurement is compared to lines of changing dermal melanin concentration with constant dermal melanin depth. The line that intersects the measurement point provides a value of dermal melanin concentration which can be used as an approximation to the value of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). Figure 13 describes this process.

In one particular implementation of the process described in figure 13 these measurements are taken over a series of adjacent 15 micron square pixels over a 10 mm area, and the spectral measurements are made are the percentage remittance of light from the tissue at three separate primary wavebands with peaks at 450nm 550nm and 650nm, each with a 80nm bandwith.

Alternatively the measured point could be plotted on figure 3 where the measurement is compared to lines of changing dermal melanin depth with constant dermal melanin concentration. The line that intersects the point provides a value of dermal melanin depth which can be used as an approximation to the value of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). In this case point 3 would represent a zero value, and point 4 a maximum value.

If an array of spectral measurements are made and a corresponding array of fn(Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration) is calculated, then a colour can be assigned to values of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) and the data represented as a false colour image.

In a further invention the approximate value of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) obtained from the above methods can be further analysed to enable suspicious lesion to be differentiated from benign ones. The following analysis technique can also be applied to arrays of other measurements made spatially across a tissue sample. In particular the measurements may be of blood or collagen concentration.

In the particular case of skin lesions, malignant skin lesions often contain the pigment melanin in the papillary dermis of the skin. A device that detects the presence of dermal melanin is effective at detecting malignant lesions and such devices are described in our patent applications GB0016690.0 and GB0112501.2. However these devices suffer from false positives generated by benign lesions which also contain dermal melanin. It is well known that several types of benign skin lesions contain dermal melanin, including the compound nevus and blue nevus. Therefore to increase the specificity of a device to detect malignant lesions a method of differentiating benign lesions containing dermal melanin for malignant lesions is beneficial.

A well known property of malignant lesions is the asymmetric and chaotic growth of the lesion. This invention is a method of automatically measuring the chaotic growth of the lesion.

Figure 7 shows a diagram of a typical skin lesion viewed from above. The skin 26 contains a malanocytic lesion 25, containing epidermal melanin as well as a region containing both epidermal and dermal melanin 27.

This invention creates two data arrays to describe the lesion derived from spectral measurements using the techniques described in the text above and our previous patents which are also detailed above.

In this particular embodiment one array contains values proportional to the total amount of melanin in the skin, and a second with values approximating to fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). Where no dermal melanin exists this second array has zero values.

A well-known property of skin lesions is they have characteristic patterns in the distribution of melanin. Benign lesions have a variety of patterns, but importantly these patterns are similar in both the epidermis and papillary dermis as melanocytes slowly descend from the dermal/epidermal junction into the papillary dermis. These melanocytes can also cease melanin production limiting dermal melanin to a shallow even layer in the papillary dermis. Malignant lesions characteristically grow chaotically in three dimensions so the patterns of melanin distribution are significantly different in the epidermis and papillary dermis. This invention automatically detects these differences through a mathematical process.

Figure 11 describes this process. In a particular implementation it operates on two arrays of data generated from spectral measurements taken over an area of skin. One array is of measurements of total melanin calculated using the method described in GB9624003.1 and PCT/GB00/02124, and the second is an array of values of fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) or 'dermal melanin volume' calculated from the process described in figure 13.

In step 1 of figure 11 the lesion is identified by applying a threshold to values of total melanin TM(x,y). In step 2 the texture of the total melanin array within the lesion is measured. In one implementation the standard deviation SD is used, but other well known statistical measures could also be used such as range or fractal dimension. In step 3 areas inside the lesion where dermal melanin is present are identified as those coordinates where the 'dermal melanin volume' DMV(x,y) is not zero. In step 4 the same texture measurement is made of DMV(x,y). In this implementation the standard deviation SD is used again, but other well known statistical measures could also be used such as range or fractal dimension. In step 5 a ratio of the two texture measurements is made and in step 6 this is threshold is applied to determine malignancy.

An improvement to this sequence is to add a preliminary step which detects elements in the array where hairs are present on the skin and marks them to be excluded from the calculation process. Erroneous data from the presence of hair has been found to disrupt this algorithm and provide unreliable results.

A particular embodiment of a device to implement the above techniques and those described in GB9624003, PCT/GB00/02124 and GB0112501.2 is described below and in figures 8 and 9. This device consists of a light tight housing 30 containing a transparent optical window 29 which is placed in contact with the skin 28. Light of a specific

wavelength band is generated by light emitting diodes mounted on a ring 33. This ring may contain a number of groups of LEDs each with it's own emission waveband. In one particular embodiment there are four groups of LEDs, each group containing between 8 and 24 individual LED dies, this number depends on the brightness of the individual devices, where relatively dim devices are used in larger numbers to approximately equalise the illumination intensity across wave bands. In one particular embodiment the wavebands of each group of LEDs span between 400nm and 1000nm. In one particular embodiment an additional reflector is mounted beside each LED to ensure a majority of the emitted light is reflected onto the window 19. In one particular embodiment an additional filter is added over one or more of the LED groups to eliminate unwanted emissions in the infra-red region of the spectrum. This ring 33 is covered by a polarising filter 32. An important feature of the design of the LED system is that the LEDs are evenly spaced relative to the window 29 to ensure even illumination of the skin 28. The LED illuminator is controlled by an electronic driver system 38 which receives control signals from the processing means 39. The electronic driver system responds to signals received from the processing means and switches the LED groups on and off in sequence as required to synchomise with the camera system 35 and 37. In one embodiment the driver system can deliver different currents to each LED group, these currents can be generated according to data passed from the processing means 39 to the LED control system 38. In one embodiment the driver system can deliver different currents to each LED to compensate for the differences in efficiency of each device. Light remitted from the skin 28 passes through a second polarisation filter 31 mounted such that its polarisation axis is at 90 degrees relative to the polarisation axis of filter 32. This light then passes into the lens 34, and is focussed onto a CCD sensor array 35 . In a particular embodiment the lens 34 is designed to focus affectively over a wide waveband corresponding to the wavebands emitted by the LED illuminator 33. In a particular embodiment this waveband is from 400 nm to 1000 nm. The CCD sensor is controlled by an electronic system 37 which converts the light intensity on each array element into a digital pixel value in an image array which is passed to the processing means 39. In a particular implementation the processing means can control the exposure time and gain of the CCD array 35. The complete device is controlled by a control programme implemented by the processing means 39. This programme provides for a sequence of images to be acquired, each with a different illumination spectrum provided by the LED light source 33 and a corresponding exposure time. In one particular embodiment eight individual images are recorded, one with each of four wavelength bands with exposures set such that normal skin can be recorded. On the three wavelength bands which lie in the visible spectrum an additional over exposed image is recorded with 4 times the standard exposure time for that wavelength band. The eighth image is taken with no illumination to determin the black values for each picture element. The image acquisition cycle is triggered by switch 36. The image data is then processed using algorithms described above. The process data is displayed on a screen 40.

One particular embodiment of this device is illustrated in figure 9, in which items 29 to 38 are contained within a handset 41 and the processing means and display means are provided by a laptop computer 42. This provides a compact and portable implemenation of this device.

In a second embodiment, illustrated in figure 10, all items 29 – 40 are integrated into a hand held battery operated device. In this embodiment the output is simplified using the method described above and in figure 11 and an output is presented a small LCD screen 43. The optical window 29 is shown as 44.

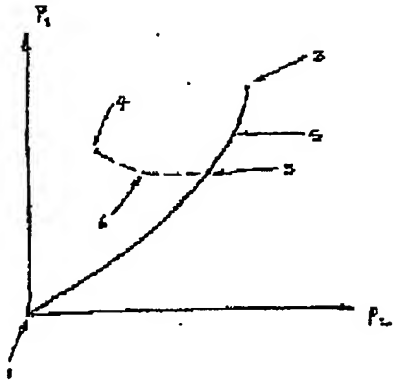

Figure 1

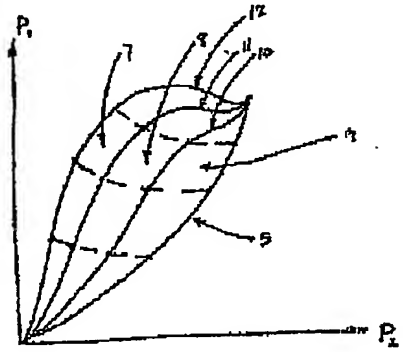

Figure 3

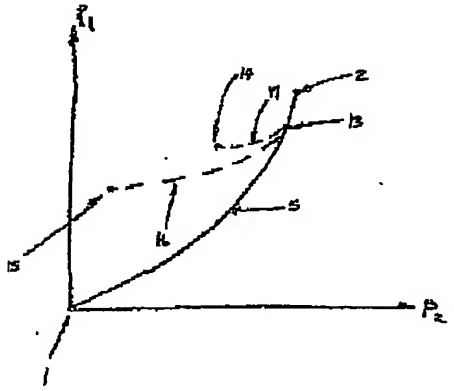

Figure 4

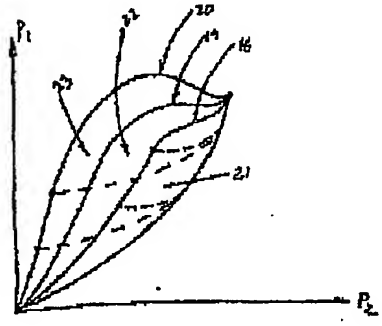

Figure 5

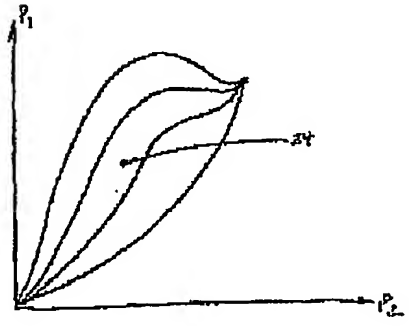

Figure 6

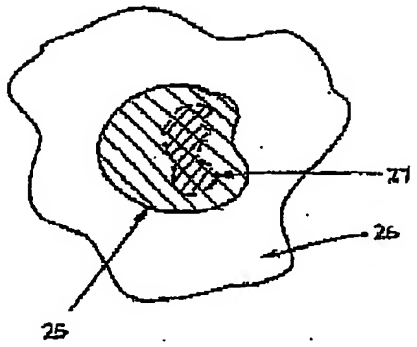

Figure 7

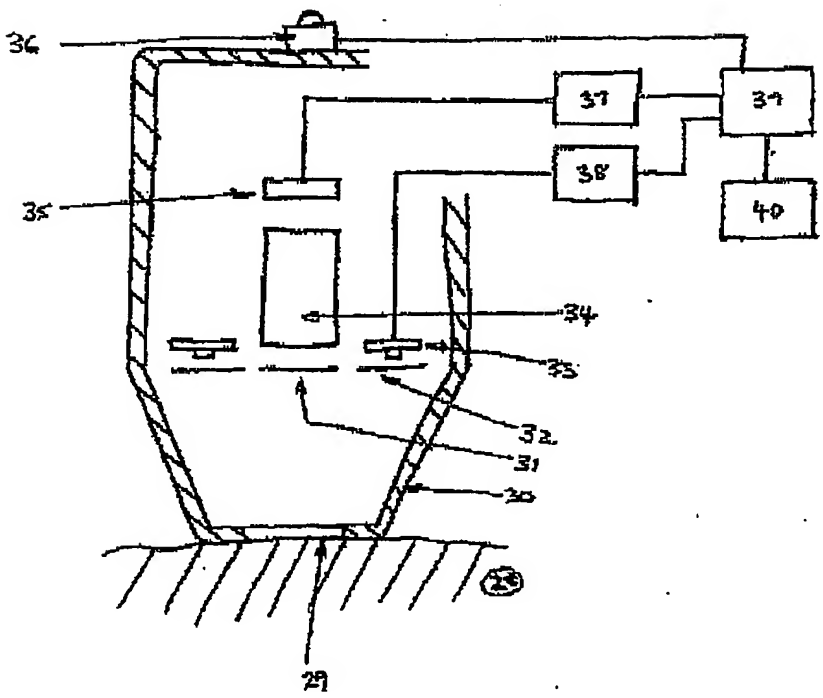

Figure 8

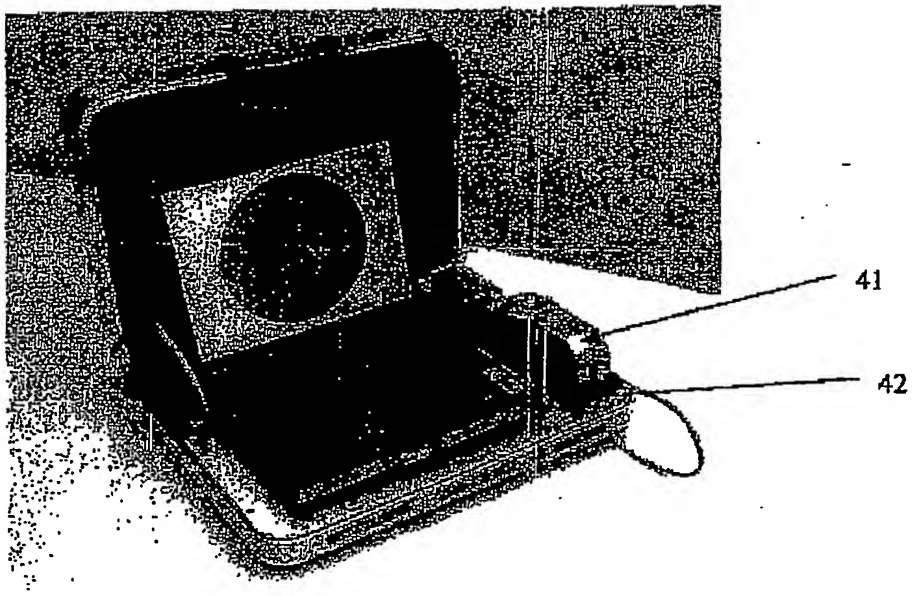

Figure 9

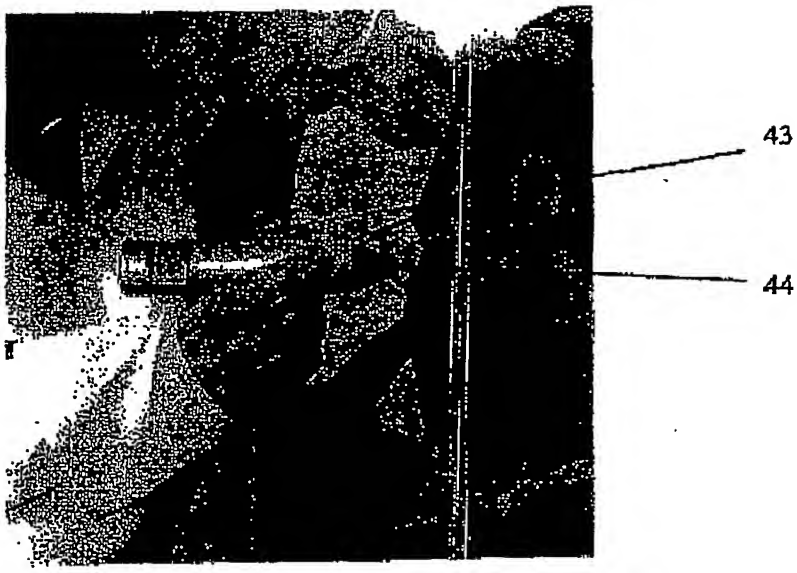

Figure 10

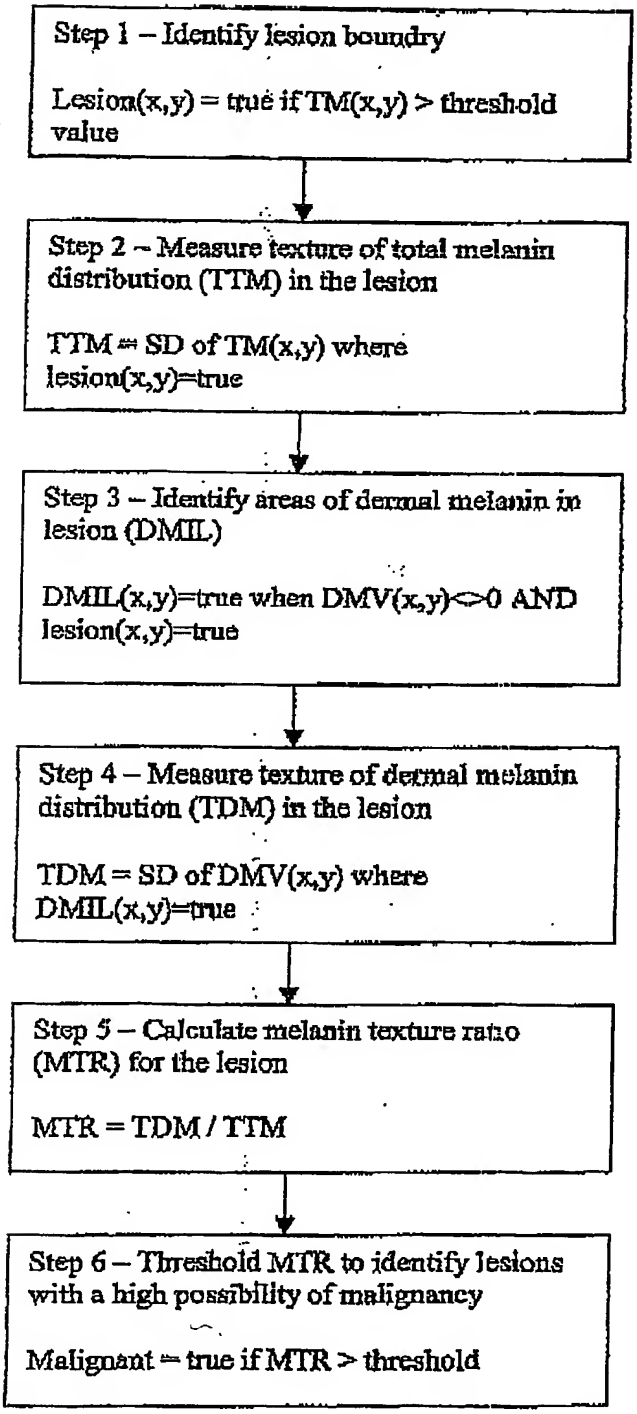
Step 1 – Identify lesion boundry
Lesion(x,y) = true if TM(x,y) > threshold value
Step 2 – Measure texture of total melanin distribution (TTM) in the lesion
TTM = SD of TM(x,y) where lesion(x,y)=true
Step 3 – Identify areas of dermal melanin in lesion (DMIL)
DMIL(x,y)=true when DMV(x,y)<>0 AND lesion(x,y)=true
Step 4 – Measure texture of dermal melanin distribution (TDM) in the lesion
TDM = SD of DMV(x,y) where DMIL(x,y)=true
Step 5 – Calculate melanin texture ratio (MTR) for the lesion
MTR = TDM / TTM
Step 6 – Threshold MTR to identify lesions with a high possibility of malignancy
Malignant = true if MTR > threshold

Figure 11

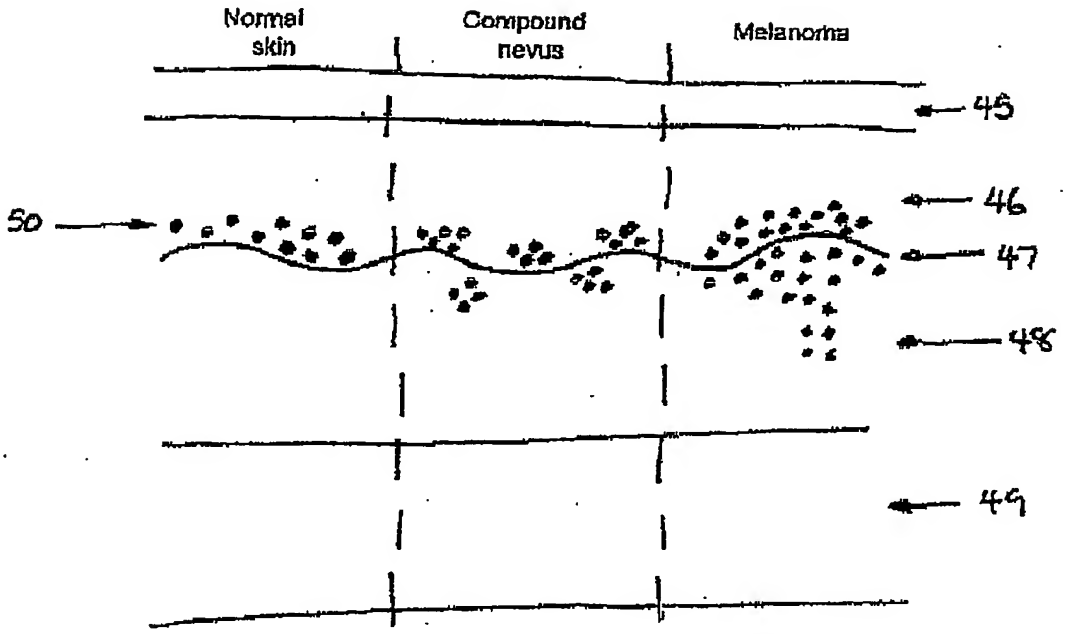

Figure 12

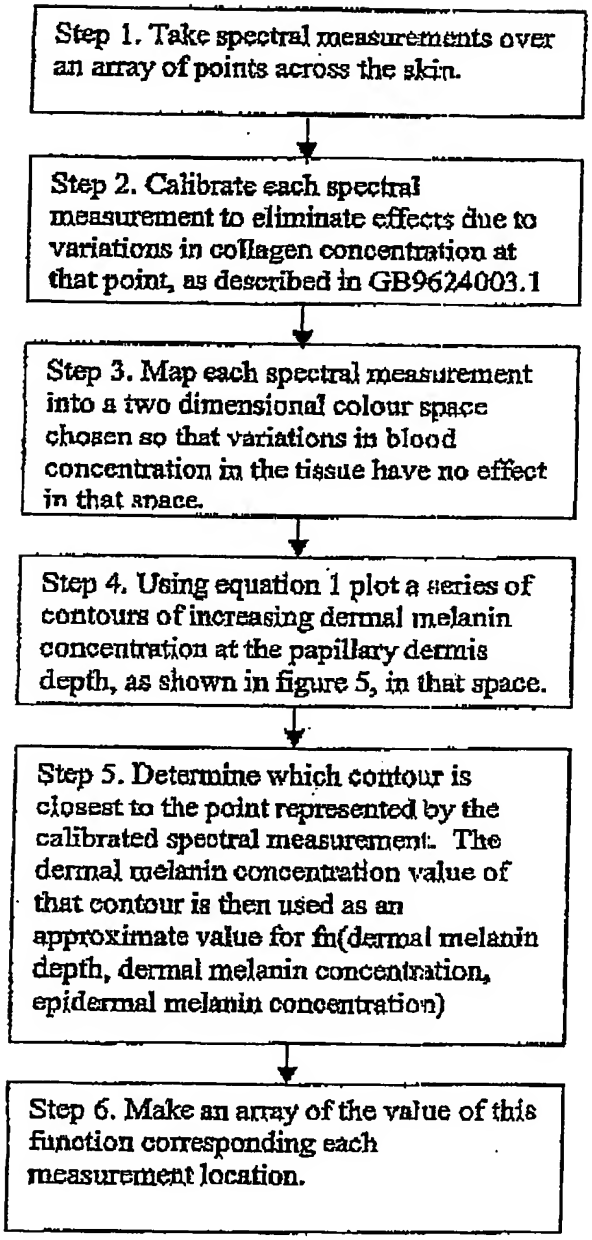
Step 1. Take spectral measurements over an array of points across the skin.
Step 2. Calibrate each spectral measurement to eliminate effects due to variations in collagen concentration at that point, as described in GB9624003.1
Step 3. Map each spectral measurement into a two dimensional colour space chosen so that variations in blood concentration in the tissue have no effect in that space.
Step 4. Using equation 1 plot a series of contours of increasing dermal melanin concentration at the papillary dermis depth, as shown in figure 5, in that space.
Step 5. Determine which contour is closest to the point represented by the calibrated spectral measurement. The dermal melanin concentration value of that contour is then used as an approximate value for fn(dermal melanin depth, dermal melanin concentration, epidermal melanin concentration)
Step 6. Make an array of the value of this function corresponding each measurement location.

Figure 13